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Award Number: DAMD17-00-1-0228

TITLE: Functional Characterization of TPF (Tumor Promoting Factor), a Novel Angiogenic Factor in Breast Cancer Pathogenesis

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REPORT DATE: June 2003

TYPE OF REPORT: Annual Summary

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	June 2003	Annual Summary (1 June 2002 - 31 May 2003)

4. TITLE AND SUBTITLE
Functional Characterization of TPF (Tumor Promoting Factor), a Novel Angiogenic Factor in Breast Cancer Pathogenesis

5. FUNDING NUMBERS
DAMD17-00-1-0228

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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES
Original contains color plates. All DTIC reproductions will be in black and white.

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT (Maximum 200 Words)

None provided

14. SUBJECT TERMS
None Provided

15. NUMBER OF PAGES
11

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

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INTRODUCTION

The primary goal of this project is to test the hypothesis that a newly identified protein, periostin, functions as an angiogenic factor to promote tumor progression and metastasis. As our previous results support this assumption, we in this final report have conducted additional experiments to generate substantial evidence for strengthening our conclusion. We wish to firmly establish an important functional role for periostin in the pathological process of breast carcinogenesis and lay the foundation for the development of novel therapeutics for the treatment of breast cancer.

BODY

Task 1: Determine periostin mRNA expression in human breast cancers.

In our last report, we have shown that periostin protein expression in human breast cancer tissues was found mainly in breast cancer cells by performing immunohistochemical analysis. To confirm that the protein was largely derived from carcinoma cells, we determined the mRNA expression in corresponding cancer tissues by using in situ hybridization and we indeed found that periostin mRNA was detected mainly in areas of breast carcinoma cells within the tumor sections but not in normal breast tissues (Figure 1). This finding demonstrates that periostin over-produced by breast cancers is predominantly derived from breast cancer cells.

Task 2: Determine the role of VEGF receptor Flk-1/KDR in periostin-induced angiogenesis.

Our previous study has highlighted that VEGF receptor-2 Flk-1/KDR, one of the major tyrosine kinase receptors mainly expressed on endothelial cells, is assigned to mediate periostin-induced tumor angiogenesis. Tumor xenografts generated from periostin-producing tumor cell lines were found to contain high vasculatures with elevated Flk-1/KDR expression. In consistent with these results, isolated recombinant periostin protein markedly increased vascular endothelial cell Flk-1/KDR expression and stimulated its angiogenic activity in vitro assays. To firmly establish the role for Flk-1/KDR up-regulation in the mediation of pro-angiogenic activity of periostin, we collaborated with Dr. Mikhail L. Gishizky in SUGEN inc. and employed two specific inhibitors of Flk-1/KDR in our functional assays. SU5416 has been previously reported to specifically block the kinase activity of Flk-1/KDR, and sFlk is a soluble form of VEGF receptor that has been demonstrated to sequester VEGF. As shown in Fig. 2A and 2B, we found that the periostin-induced increases in cellular migration and tube formation in matrigel by the HMVEC (human microvascular endothelial cell) were significantly inhibited by the presence of

those two inhibitors. Most importantly, the increased tumor growth resulted from the production of periostin was completely reversed by the presence of the inhibitor SU5416 in our xenograft model system (Fig. 3A). This notion was further supported by the reduction in hemoglobin content (Fig 3B), anti-CD31 staining (vessel marker protein) and anti-Flk-1/KDR staining in tumor sections as a result of SU5416 treatment (Fig. 4). In aggregate, these data strongly support the conclusion that the up-regulation of Flk-1/KDR expression and consequently the sensitization of endothelial cells to the potent angiogenic factor VEGF is at least partially responsible for the mediation of periostin-induced tumor angiogenesis.

Task 3: Identify periostin-activated integrin $\alpha_v\beta_3$-FAK signaling pathway.

Finally, we investigated which signaling pathway activation is mainly mediated in periostin-prompted Flk-1/KDR up-regulation. A recent report in literature suggested that periostin could functionally interact with integrins to mediate the adhesion and migration of human ovarian carcinoma cells. To test the possibility that periostin may induce Flk-1/KDR expression through interaction with integrins in endothelial cells, we examined the profile of integrin expression in HMVEC and found those cells to express predominantly $\alpha_v\beta_3$ integrins (Fig. 5A). We next probed if interference with the function of integrins using specific anti-integrin antibodies has an effect on the ability of periostin to induce the expression of Flk-1/KDR in HMVEC. As shown in Fig. 5B, treatment of HMVEC with periostin in the presence of anti-$\alpha_v\beta_3$ integrin antibody prevented the induction of Flk-1/KDR. The specificity of this blockage of periostin activity by interfering with the function of $\alpha_v\beta_3$ integrin was demonstrated by the lack of an effect on the periostin mediated induction of Flk-1/KDR expression when anti-$\alpha_v\beta_5$ integrin antibody was used in the same assay. The initial step of integrin signaling involves the activation of focal adhesion kinase (FAK). Consistent with this notion, we found that transient stimulation of HMVEC with periostin augmented the phosphorylation of FAK on tyrosine 681 (Fig. 5B), an event indicative of activation of FAK. The increase in FAK phosphorylation on Tyr681 was reversed to the basal level by the presence of anti-$\alpha_v\beta_3$ integrin antibody but not the anti-$\alpha_v\beta_5$ integrin antibody. Taken together, these results strongly suggest that the $\alpha_v\beta_3$ integrin-FAK signaling pathway plays an essential role in mediating the effect of periostin on the up-regulation of Flk-1/KDR expression in HMVEC.

KEY RESEARCH ACCOMPLISHMENTS

1. The findings strongly support our hypothesis that overexpression of a mesenchymal gene, periostin, by breast epithelial carcinoma cells may confer growth advantage in tumor development in vivo through the promotion of angiogenesis.

2. Our results disclosed that periostin prompts tumor angiogenesis, at least in part, by up-regulation of VEGF receptor in endothelial cells.

3. The data revealed the integrin $\alpha_v\beta_3$-FAK signaling pathway is required for periostin effect on regulation of Flk-1/KDR expression in endothelial cells.

REPORTABLE OUTCOMES

This findings combined with previous data are issued in a manuscript listed below.

Shao, R., Bao, S., Bai, X., Blanchette, C., Anderson, R. M., Marks, J. R., Gishizky, M. L., Wang, X.-F. Acquired expression of periostin by breast cancers promotes tumor progression via enhancement of angiogenesis. Submitted.

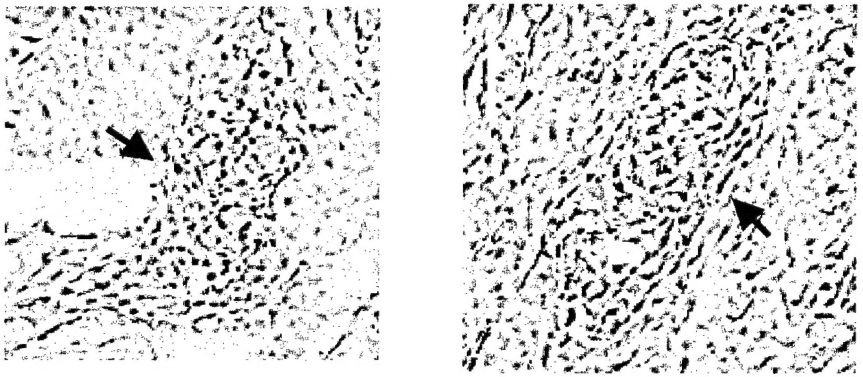
Partial of this work was selected for a Symposium Platform Presentation at the Era of Hope Department of Defense Breast Cancer Research Program Meeting to be held in Orlando in September 2002.

Principle investigator was supported by this grant: Rong Shao, Ph.D.

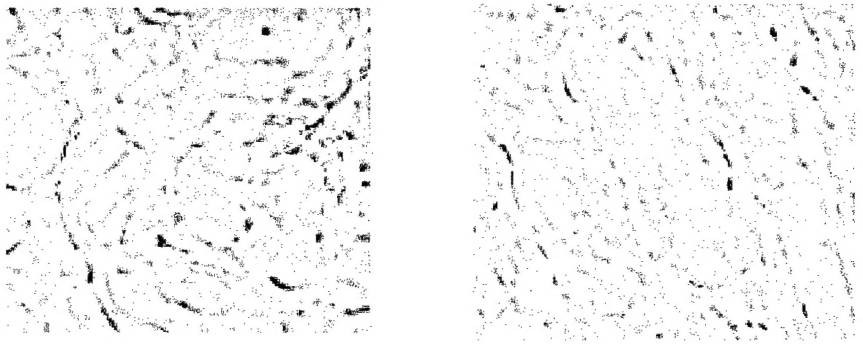
CONCLUSIONS

In this final report, we describe our research accomplishment in confirming periostin angiogenic function and molecular mechanism. The important role of Flk-1/KDR in periostin-induced angiogenesis was demonstrated by employing the receptor inhibitors in cell culture condition and tumor xenografts in nude mice. The signaling pathway involved in up-regulation of Flk-1/KDR was found the activation of integrin $\alpha_v\beta_5$-FAK cascade. This identification of molecular mechanism for periostin angiogenic function provides a novel insight into the mechanistic basis for human cancers during later stages of tumor progression and also provides valuable information for therapeutic application in halting cancer development.

Breast cancer



Normal breast tissue



in situ hybridization

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figure1

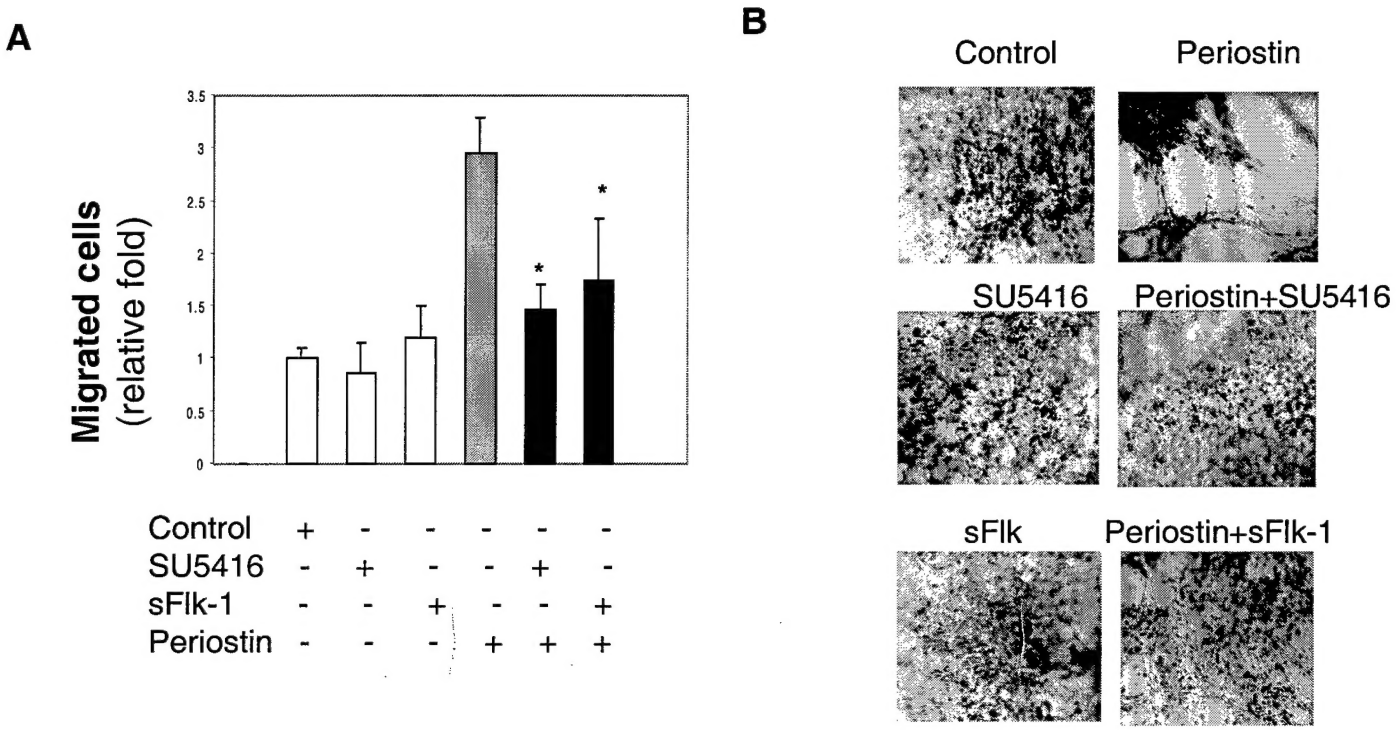

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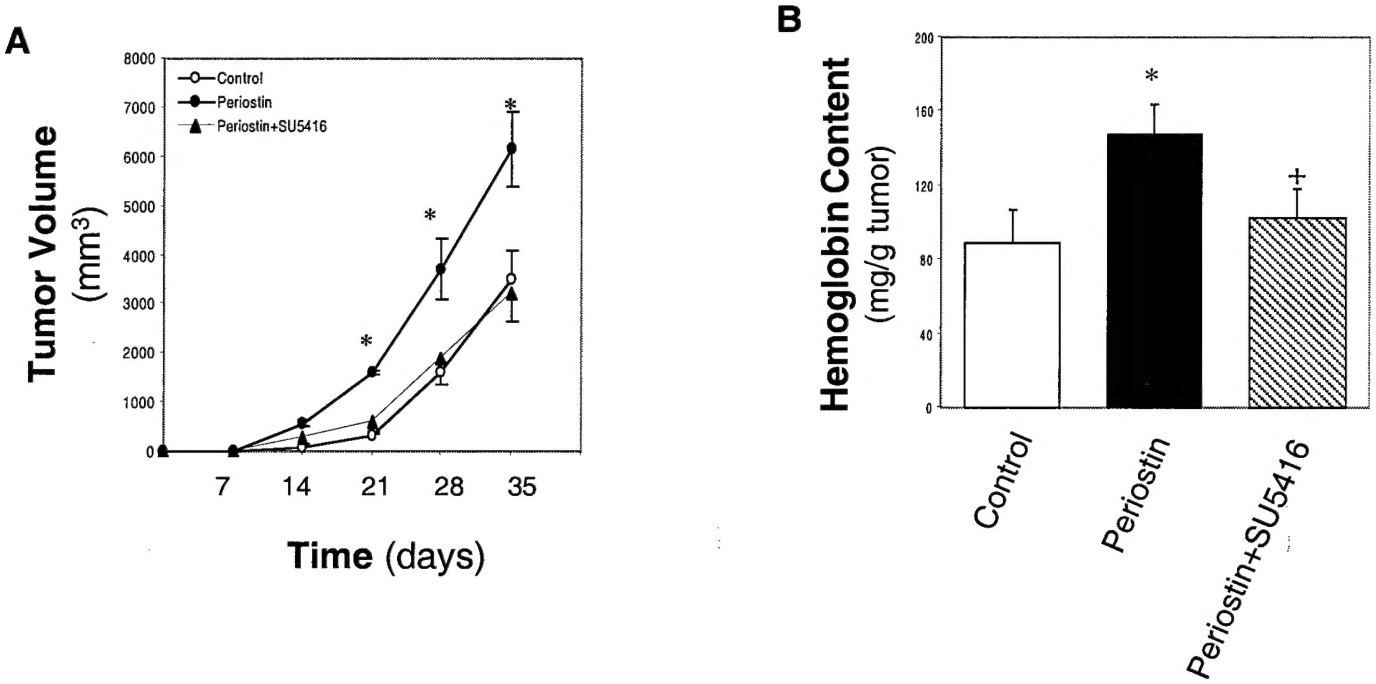
Figure 2



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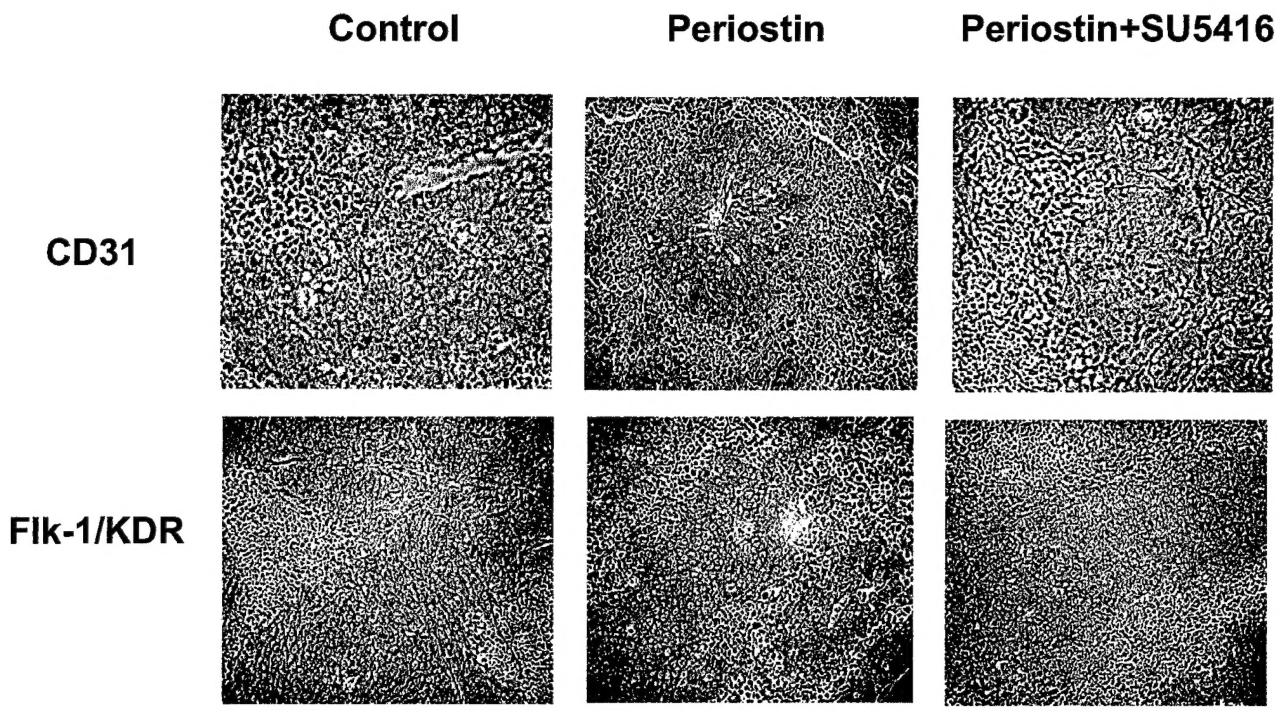

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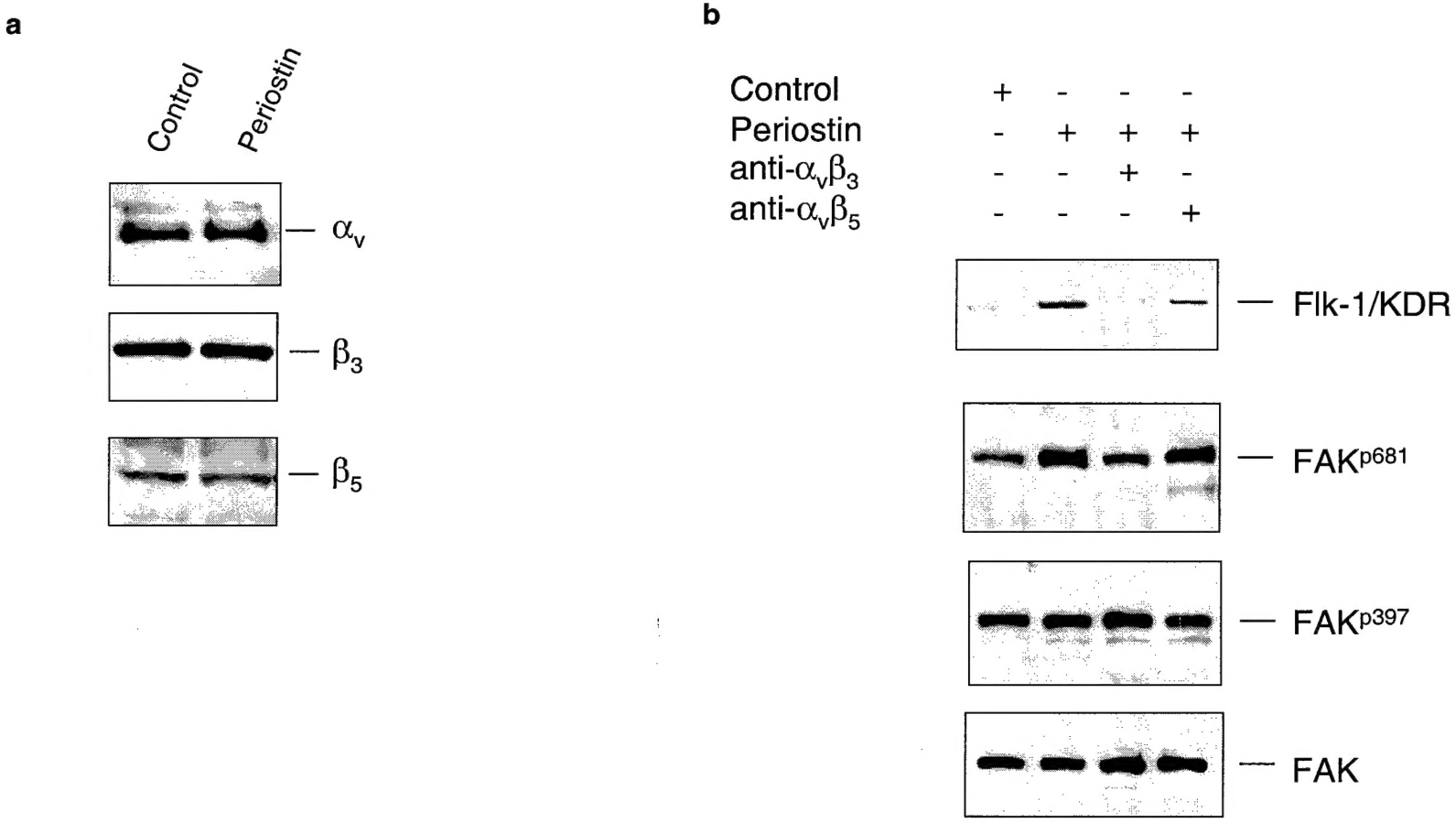

Shao
Figure5